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Catalytic Center Assembly of HPPK as Revealed by the Crystal Structure of a Ternary Complex at 1.25 A Resolution

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ABSTRACT: Folates are essential for life. Unlike mammals, most microorganisms must synthesize folates de novo. 6-Hydroxymethyl-7, 8-dihydropterin pyrophosphokinase (HPPK) catalyzes pyrophosphoryl transfer from ATP to 6-hydroxymethyl-7,8-dihydropterin (HP), the first reaction in the folate pathway, and therefore is an ideal target for developing novel antimicrobial agents. HPPK from Escherichia coli is a 158-residue thermostable protein that provides a convenient model system for mechanistic studies. Crystal structures have been reported for HPPK without bound ligand, containing an HP analog, and complexed with an HP analog, two Mg(2+) ions, and ATP. We present the 1.25 A crystal structure of HPPK in complex with HP, two Mg(2+) ions, and AMPCPP (an ATP analog that inhibits the enzymatic reaction). This structure demonstrates that the enzyme seals the active center where the reaction occurs. The comparison with unligated HPPK reveals dramatic conformational changes of three flexible loops and many sidechains. The coordination of Mg(2+) ions has been defined and the roles of 26 residues have been derived. HPPK-HP-MgAMPCPP mimics most closely the natural ternary complex of HPPK and provides details of protein-substrate interactions.